

INSIDE THE BIODEGRADATION PROCESS IN CONTAMINATED GROUNDWATER

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14th of May 2013

ATWARM Conference “Water - The Greatest Global Challenge”
Dublin City University

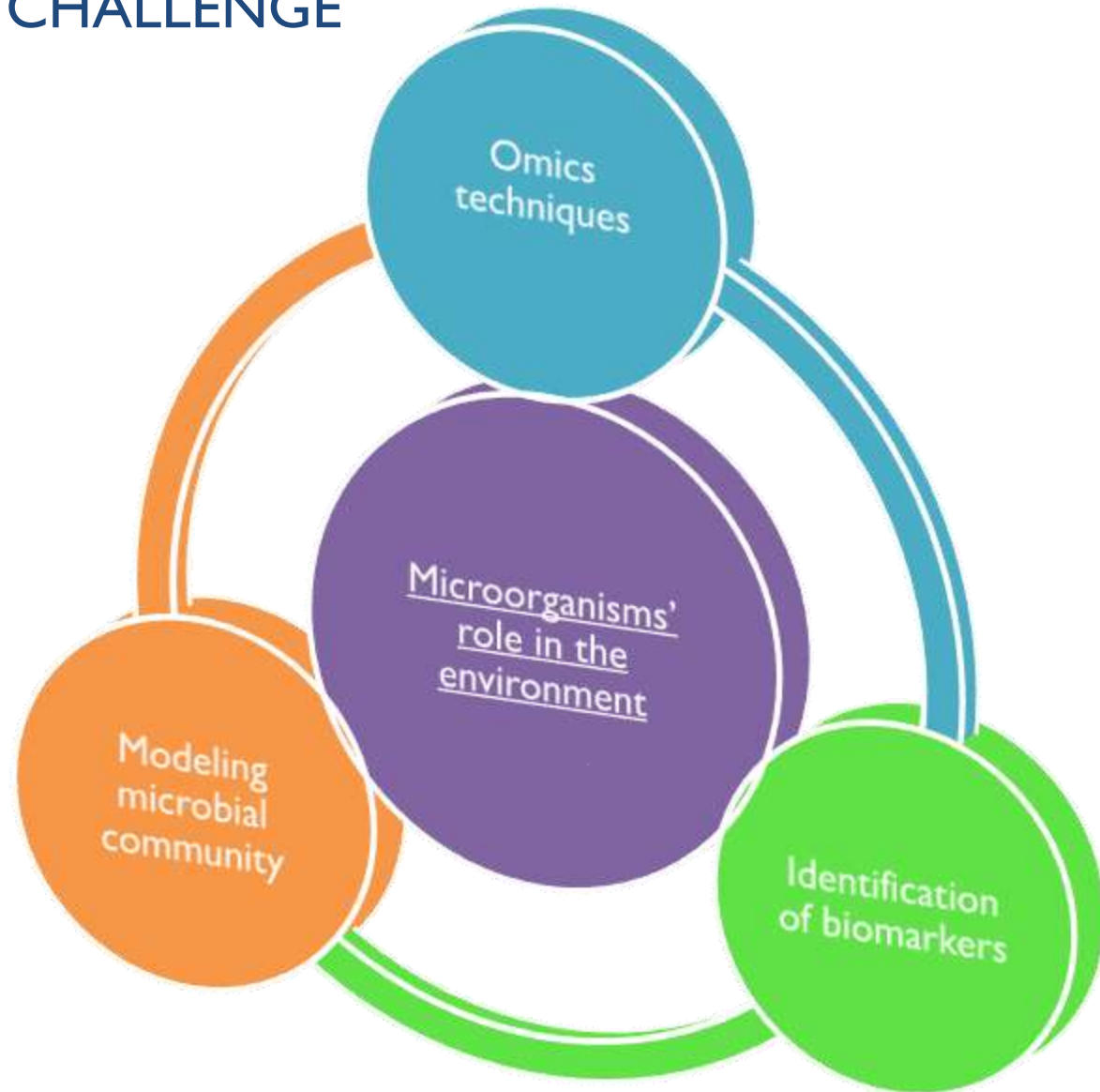
BIOREMEDIATION & BIODEGRADATION

- Use of microorganisms to recover contaminated environment
- Exxon-Valdez – Alaska 1989
Bioremediation main method used (biostimulation through the addition of fertilizer)¹
- BP Deepwater Horizon – Gulf of Mexico 2010 molecular biological techniques (e.g. microarray) immediately used to understand the role of microorganisms in the degradation process¹



¹ Atlas R. M., Hazen T. C. 2011 “Oil Biodegradation and Bioremediation: A tale of the Two worst Spills in U.S. History” *Environmental Science & Technology*, 45, 67067-6715

BLACK BOX CHALLENGE



OUR CONTRIBUTION...

- Microbial community study in contaminated groundwater
- Functional genes sequencing and qPCR
- Identification of new biomarkers
- Development and update of rapid methods to assess the biodegradation potential in contaminated groundwater

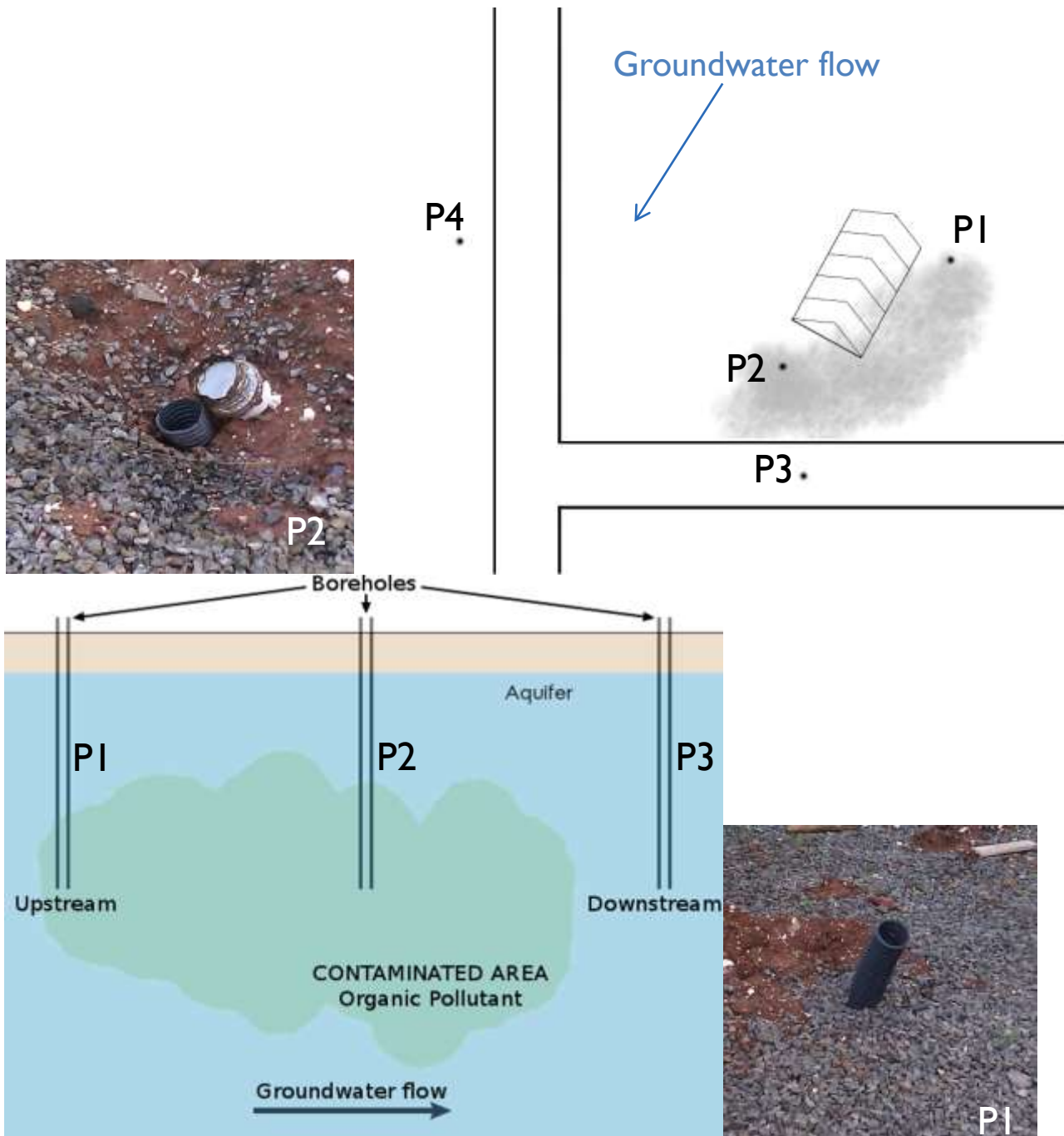


CONTAMINATED SITE

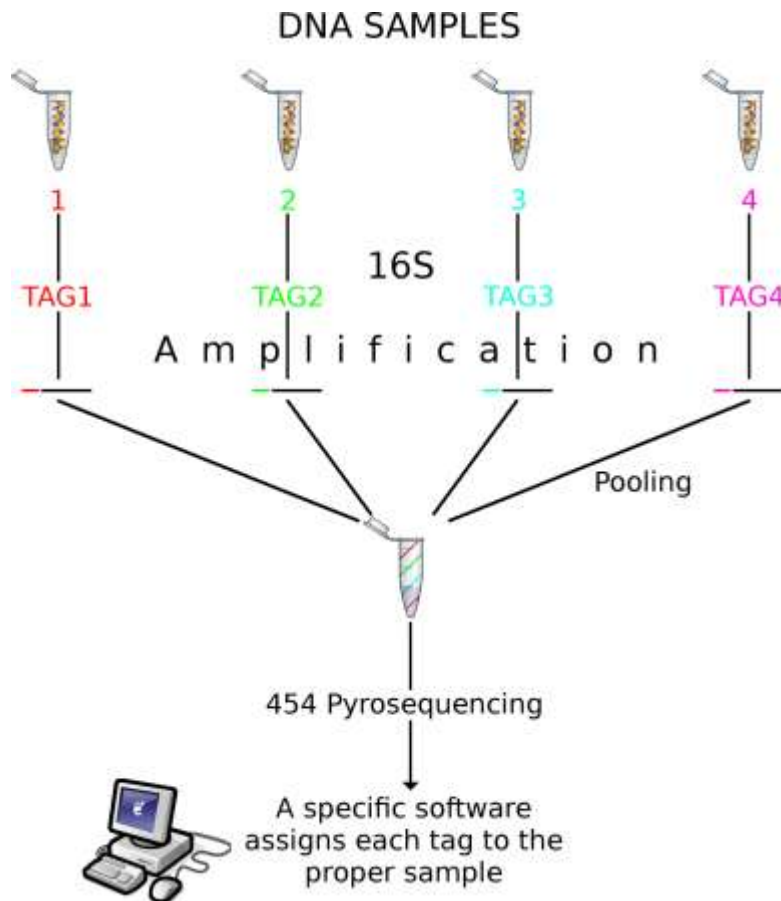
- Area located in Northern Ireland, contaminated by Diesel after an accidental spillage.

- Contamination level: Aliphatic C5-C35 480 ppm, Aromatic C5-C35 130 ppm.

- Negative redox potential and lower DO in P1, P2 and P3 but not in P4 (clean control).



MICROBIAL COMMUNITY STUDY: 16S rRNA GENE BARCODED PYROSEQUENCING

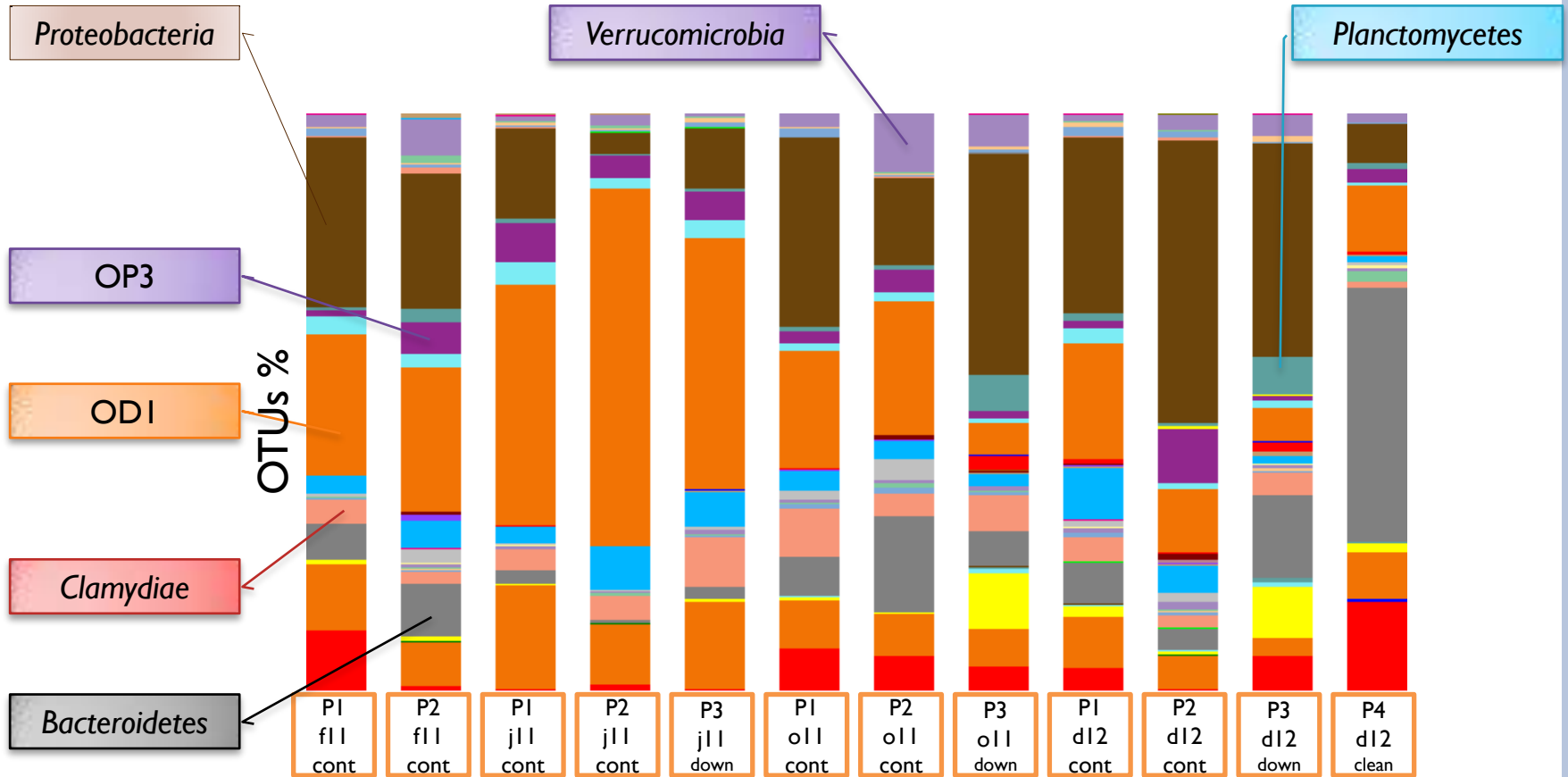


Data Analysis:

- QIIME Pipeline¹
- USEARCH: noise removal, chimera detection (UCHIME) and OTUs picking^{2,3}
- Pynast: alignment⁴
- GreenGenes 10_12 and RDP Classifier 2.2: assign taxonomy^{5,6}
- Fast Tree 2.1.3: phylogenetic tree⁷

¹ Caporaso JG, et al. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7:335-336. ² Edgar RC. **2010**. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26(19):2460-2461. ³ Edgar, RC et al. **2011**. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* btr381. ⁴ Caporaso JG, et al. **2010**. PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* 26:266-267. ⁵ McDonald D et al **2012**. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J* 6(3): 610–618. ⁶ Wang Q et al. **2007**. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microb* 73(16): 5261-5267. ⁷ Price MN, Dehal PS, Arkin AP. **2010**. FastTree 2-Approximately Maximum-Likelihood Trees for Large Alignments. *Plos One* 5(3).

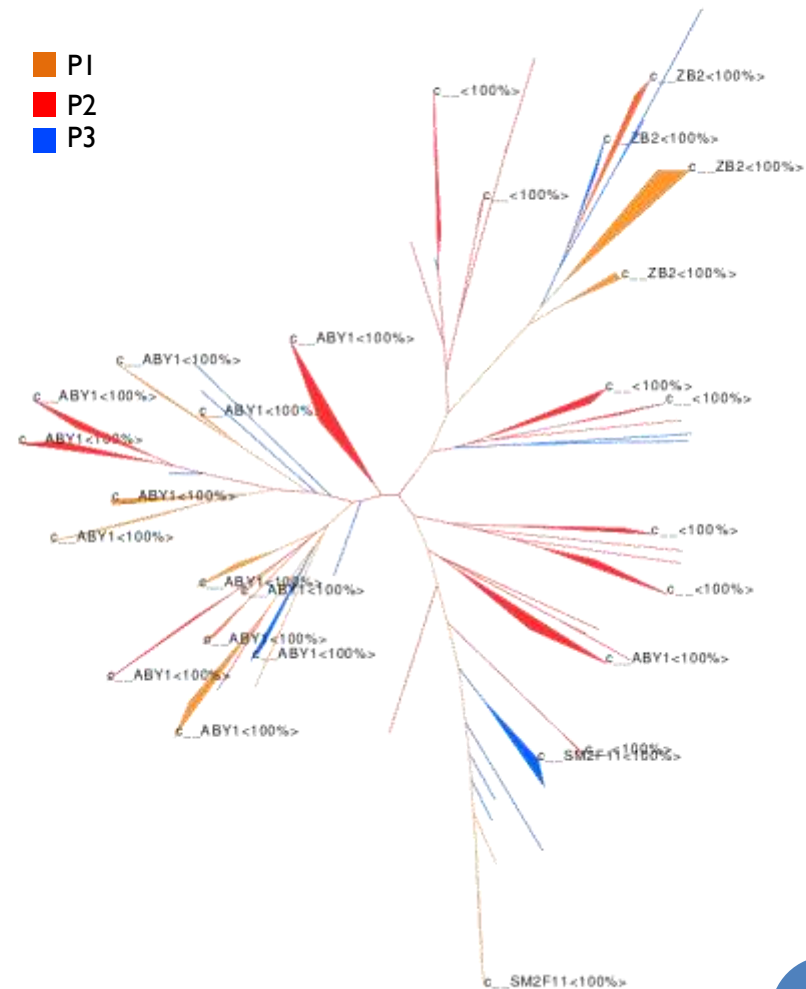
MICROBIAL COMMUNITY STUDY: TAXA SUMMARY AT *PHYLUM* LEVEL



49% of Proteobacteria:
34.8% of Synthrophobacterales
fermentative bacteria!!

MICROBIAL COMMUNITY STUDY: CANDIDATE DIVISION ODI

- Never isolated in pure culture
- Strictly anaerobe, fermentative, non respiring, produces acetate, generates and consumes H₂ and/or SH₂
- Widespread in aquatic environments
- Already detected in contaminated groundwater

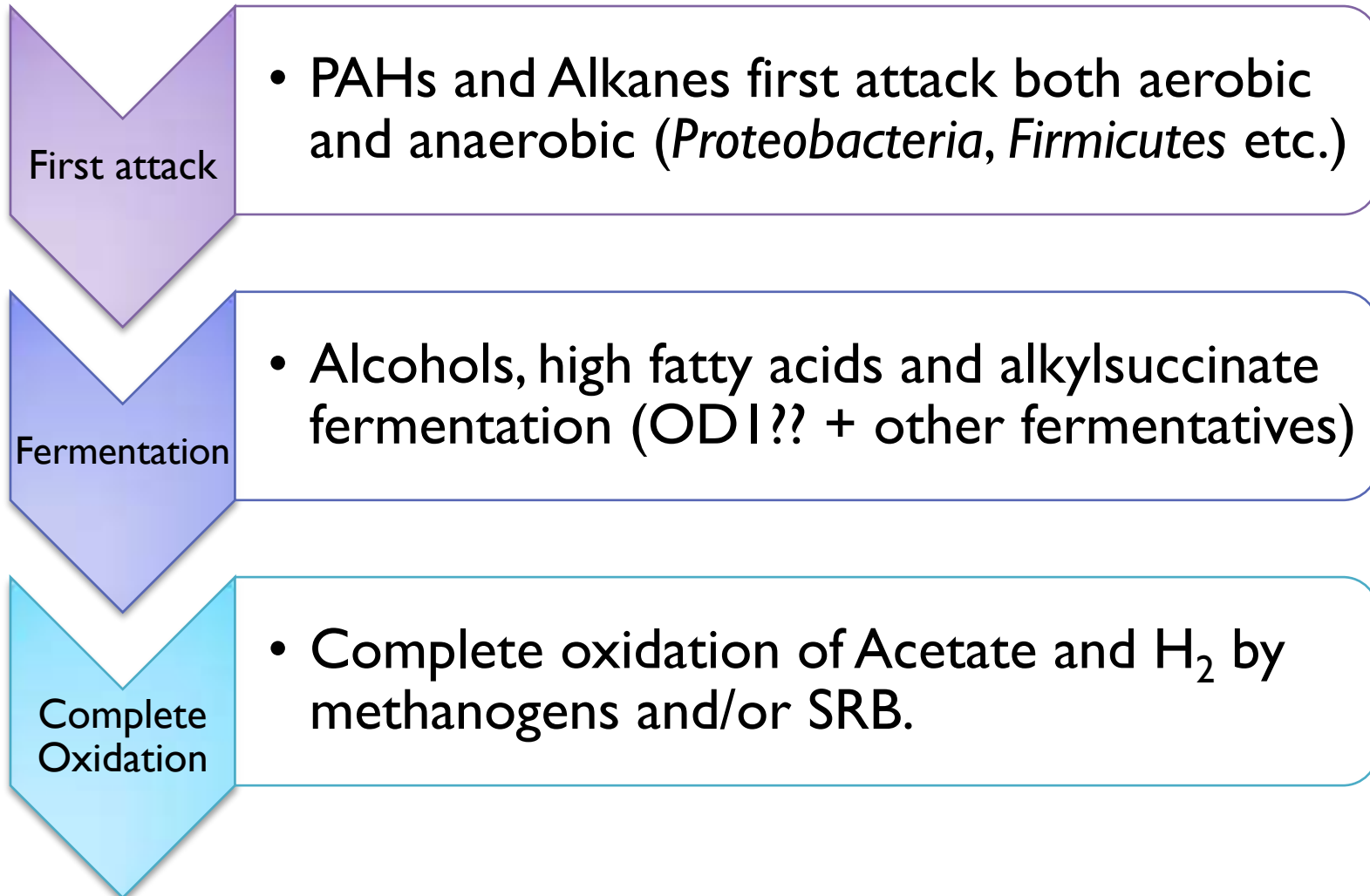


ODI: Wrighton KC et al. **2012**. Fermentation, Hydrogen, and Sulfur Metabolism in Multiple Uncultivated Bacterial Phyla. *Science* 337:1661-1665.

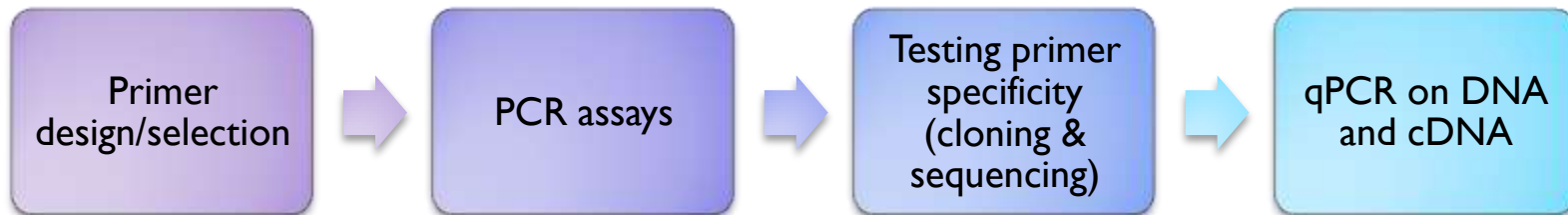
Tree Software: Pirrung M et al. **2011**. TopiaryExplorer: An application for connecting large phylogenetic trees to environmental metadata; *Bioinformatics* 27(21): 3067–3069.

WHAT ABOUT THE BIODEGRADATION PROCESS?

OUR HYPOTHESIS...



WORKFLOW: ASSESSING THE BIODEGRADATION PROCESS



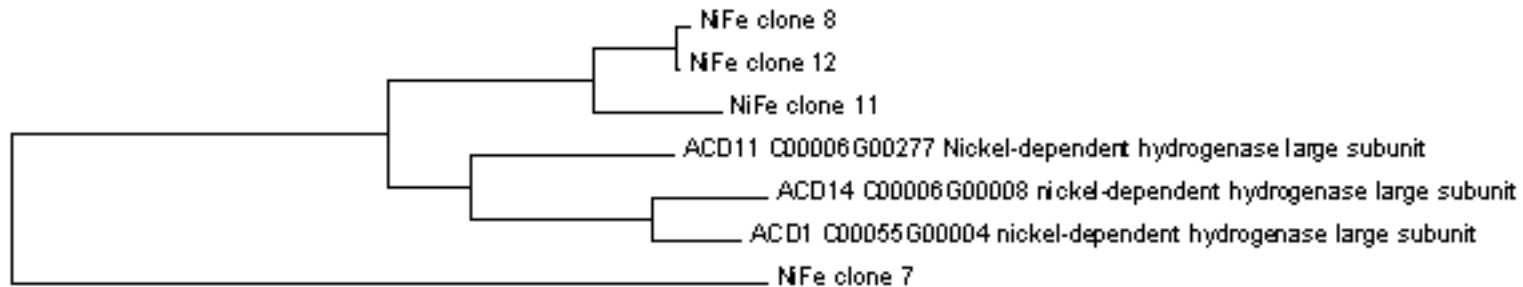
FUNCTIONAL GENES

Step	Pathway	Enzyme	Primers	PCR assays				Primers tested (sequencing)
				P1	P2	P3	P4	
First Attack	Aerobic PAH	Dioxygenase GRAM +	PAH-RHD GP (Cebron et al 2008)	+/-	+/-	-	+/-	specific
	Aerobic PAH	Dioxygenase GRAM -	PAH-RHD GN (Cebron et al 2008)	+/-	+	-	-	specific
	Aerobic Alkane	monooxygenase	AlkBw (Wang et al 2010)	+	+/-	-	-	Not specific
	Anaerobic aromatic	bamA hydrolase	SP9/ASPI (Abu Laban et al 2010)	+	+	-	+/-	specific
	Anaerobic aromatic	bamB	BamB (Loeffler et al 2011)	+/-	+	-	-	specific
Fermentation	General	Fe Hydrogenase	hydA1290F/1538R (Pereyra et al 2010)	+	+	-	-	specific
	ODI	NiFe Hydrogenase	NiFe Ia/NiFe I this study	+/-	+	-	-	specific
Complete Oxydation	Methanogens	mcrA	mcrA 1035F/1538R (Pereyra et al 2010)	+	+	-	-	specific
	SRB	dsrA	dsrA 290F/660F (Pereyra et al 2010)	+	+	-	-	specific

FOCUSING ON ODI ROLE

Identification of functional gene involved in the fermentation process (NiFe hydrogenase)

Primer design using sequences from published



Quantification + linking identity and functionality

NEW MARKER OF THE BIODEGRADATION PROCESS

IN CONCLUSION...

- Evaluation of the microbial community in contaminated groundwater: extremely informative!!
- Hypothesis of a biodegradation process mainly anaerobic, with an important contribution of fermentative bacteria!
- Identification of a new biomarker!
- In progress is the development of a rapid technology based mainly on qPCR to evaluate the biodegradation process in contaminated groundwater, validated through the comparison of DNA vs cDNA data and contaminated vs control samples data.

ACKNOWLEDGMENT

- *Whiteford Geoservices* for the samples and preliminary chemical data
- My supervisors: Prof M J Larkin, Dr L Kulakov and Dr C C R Allen for their help and support!
- Blathnaid Mc Polin for her help in samples collection and analysis of water quality parameters *in situ*
- Dr Anna Kulakova and Dr Paul Flanagan for their help in the lab
- All my colleagues of the 0G414 lab!

**Thank you for your kind
attention!**